9 Detection of a novel complex allele c.[869+11C>T;3909C>G] in CFTR gene

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Objective: The c.3909C>G (N1303K) is the most frequent mutation after the c.1521_1523delCTT (F508del) in Lebanon. A *CFTR* gene screening was performed on 10 Lebanese and 5 French CF patients carrying c.3909C>G. Parental studies revealed that all individuals have an association of c.3909C>G (exon 24) with c.869+11C>T (intron 7) in cis, inducing a new complex allele (CA). Since both mutations are located in two distant regions, we studied their combined impact by two plasmid constructions.
Method: Firstly, we studied the impact of c.3909C>G on alternative splicing (AS), localization and maturation of the CFTR protein. Secondly, to study the impact of c.869+11C>T on AS, an *ex-vivo* study will be conducted using a plasmid containing the exon 7 and its flanking introns. After transfection of different type of eukaryotic cells, RNA extraction and RT-PCR, cDNA will be sequenced to verify the possible AS.
Results: We showed that c.3909C>G affects the localization and the process. The *in-silico* study is in favor for a possible role of c.869+11C>T in AS. In fact, the used algorithm *human splicing Finder* showed that c.869+11C>T might affect the AS since it is located near the donor site.
Conclusion: The class II mutation, c.3909C>G, induces mild phenotype with few CFTR on membrane. We suggest that the CA c.[869+11C>T;3909C>G], will act like a class I mutation, explaining the severe phenotype in some c.3909C>G patients. So, the classification of a mutation is not sufficient in a clinical approach, as the possible presence of a CA may alter its the specific effect. Therefore, an individualized approach is required to perform proper diagnostic and treatment when available.

10 The role of complex alleles in patients with cystic fibrosis and L997F

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Objectives: L997F is causative of Cystic Fibrosis (CF) classic form when it is in cis with R117L mutation. In order to assess clinical characteristics and genotype of patients carrying L997F we retrospectively evaluated a cohort of CF patients in follow-up at two CF care Centers.
Methods: Complex alleles such as L997F- R117L were searched by CFTR sequencing analysis. For all patients included in the study (mean age: 26 years, range 4 months – 49 years) data were collected as age and symptoms at diagnosis, chloride level at sweat test, sputum, radiological features, pancreatic status and forced expiratory volume in one second (FEV1). L997F mutation has been detected in 19/505 (3.7%) patients; 18/19 are compound heterozygous for other CFTR causing mutations. No detection of R117L mutation in cis or other complex alleles has been found. Mean age at diagnosis is 16.8 years (range 4 months-37 years). 10/19 were diagnosed by neonatal screening, 3/19 by respiratory symptoms, 2/19 by recurrent pancreatitis and 4/19 by familiarity. All patients are pancreatic sufficient. At diagnosis sweat test was negative/borderline in 18/19 cases. Patients with a longer follow up showed a slower progression of lung disease and no chronic infections by *Pseudomonas aeruginosa*. The only homozygous patient for L997F (age 18.5 years) shows chronic bronchitis, nasal polyposis and a borderline sweat test (Cl^- 58 mEq/L)
Conclusion: L997F mutation could be responsible of a variable phenotype of CFTR-related disorders despite the absence of R117L mutation in cis.
A regular follow up could be mandatory to define the role of L997F and the inclusion of this mutation of unclear significance in screening panels.

11 A new multiplex PCR method for the quantification of aberrant transcripts from nasal epithelial cells of patients

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The in-depth analysis of the *CFTR* gene, that consists in exploring all the coding exons their flanking regions and targeted intronic sequences, leads to the frequent identification of private point mutations in patients. It is difficult to establish *a priori* the effect of rare intronic, synonymous or missense variants on the function of the encoded protein and subsequently their involvement in the disease. Consequently, we previously designed a classification method for the evaluation of their impact on splicing which provides strong arguments to select variants that require further analysis of nasal epithelial cell transcripts in the patients. For this purpose, we have now developed a multiplex fluorescent PCR for the one-step analysis of alternative and/or aberrant splicing of CFTR mRNA. We designed four different mix, using FAM-labelled forward primers, that contain the whole *CFTR* transcript in 26 overlapping fragments. We also included an internal control amplifying a sequence of the *ALAS1* transcript. PCR products were then analysed by relative quantification after capillary electrophoresis migration on the 3130XL Genetic Analyzer. In a first step we have set the optimal conditions for a balanced amplification of each amplicon using mRNA from T84 cells, known to express *CFTR*. The validation of this method is underway using mRNA from nasal epithelial cells of healthy individuals and patients carrying an identified splicing mutation. By quantifying aberrant splicing effect, this method should play its full role in the characterization and classification of rare genomic variants of unknown clinical significance.

12 Using a mRNA-based approach to detect rare CFTR mutations

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Due to the large extension of CFTR gene (~190kb) and the large number of gene variants reported (>1900) there's a need to establish quick methods for mutation analysis. Usually this is achieved by limiting analysis to the most common mutations. However, this often leads to difficulties in CF genotype identification, especially in populations with high prevalence on non-p.F508del mutations.
We have designed a mRNA-based protocol using just 9 RT-PCR reactions, hereby the complete CFTR coding region is analysed. Moreover, one of the reactions (exons 11–13) is based on ARMS for p.F508del mutation, which allows easy detection of this mutation.
We have applied this protocol to genotype 12 CF patients with absence or just residual CFTR-mediated Cl^- secretion in rectal biopsies and only 1 CFTR mutation identified by routine methods.
Using this strategy we detected mRNA alterations in 75% of the patients and identified the 2nd mutation, confirmed by genomic DNA sequencing. For 2 siblings we detected a cryptic exon between exons 10–11 resulting from a mutation far deep into IVS 10 (c.1584+18672A>G), unlikely to be detected by CFTR exon sequencing. The other mutations detected were: p.G576A, c.1717–1G>A, c.1812–1G>A, c.3272–26A>G, c.3120+1G>A (2P) (legacy nomenclature).
We conclude that this is a rapid, robust and inexpensive method to detect rare mutations that can be easily used after a first screen. Furthermore it already demonstrates the functional consequences of mutations in case they occur at the RNA level.
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